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Genetic diversity and demographic history of *Dolichus halensis* (Schaller) (Coleoptera: Carabidae) populations in the Qinling Mountains, China

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Abstract: *Dolichus halensis* (Schaller) (Coleoptera: Carabidae) is an important predator insect that is widely distributed in China. To reveal the genetic diversity and demographic history of *D. halensis* populations from the Qinling Mountains, a 1 601 bp fragment from the mtDNA gene (Cox1-tRNALeu-Cox2) was sequenced for 191 individuals from 24 local populations. Forty-five polymorphic sites were found, and 53 haplotypes were identified. The high haplotype diversity (H_d = 0.796) was accompanied by lower nucleotide diversity (P_i = 0.0033). Phylogenetic analysis (Bayesian inference) of the 53 haplotypes revealed two major clades. AMOVA analysis suggested that most of the variation occurred within populations (86.61%). SAMOVA and PERMUT analyses showed no phylogeographic structure. The results of neutrality tests and mismatch distribution analyses showed a sudden population expansion. The above analyses confirmed the existence of a postglacial population expansion.

Key words: *Dolichus halensis*; mitochondrial DNA; genetic diversity; population expansion; Qinling Mountains

1 INTRODUCTION

The Qinling Mountains are located in the central part of China (Fig. 1). According to the zoogeographical regions of China (Zhang and Zhao, 1978), they form the boundary between the Oriental Realm and the Palaearctic Realm and are the watershed for the Yangtze and Yellow River catchment areas, forming a barrier between the southern and northern parts of China. They thus resulted different climates and flora and fauna compositions (Liu *et al.*, 2004; Ma, 2007). Oriental and Palaearctic species congregate in the area, forming a specific biotic region. The Qinling Mountains are one of the centers of the origin and evolution of biodiversity in China (Guo *et al.*, 2000), with high biodiversity of both animal and plant species (Zhang and Li, 1997; Guo *et al.*, 2000). A complex, microhabitat-rich topography could also affect the genetic diversity and phylogeographic structure of animal habitats in these areas (Li *et al.*, 2012).

Similar to other regions in the northern hemisphere, the Qinling Mountains have also experienced several glacial-interglacial cycles (Tian and Huang, 1990). Past climatic events such as the Quaternary glaciation have left clues to the geographical distribution of genetic diversity in natural populations (Hewitt, 1996, 1999, 2000; Taberlet *et al.*, 1998; Avise, 2000; Carstens *et al.*, 2005). The founder effect leads to a reduction in the genetic diversity of the natural population (Hayes and Harrison, 1992; Hewitt, 2004), and the subsequent rapid natural population expansion may erase the previous geographical differences in genetic diversity (Lessa *et al.*, 2003).

The present study aims to explore the effect of the Quaternary glacial actions on the phylogeographical pattern and population expansion of the ground beetle *Dolichus halensis* (Schaller) (Coleoptera, Carabidae), which is widely distributed in Eastern Asia and Europe, in the Qinling mountainous area (Liang and Yu, 2000). The published papers on this species have mainly focused on the faunal composition (Liang and Yu, 2000) and its biological characteristics (Chen and Liu, 1992). In this study, mitochondrial DNA (Cox1-tRNALeu-Cox2) was used as a molecular marker to examine the history and geographical distribution of *D. halensis* genetic diversity in the Qinling Mountain area.

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2 MATERIALS AND METHODS

2.1 Sampling, DNA extraction, PCR amplification and sequencing

A total of 191 ground beetle *D. halensis* adults were collected from 24 locations in the Qinling Mountains from 2009 to 2010 (Table 1, Fig. 1). The sample size for each location ranged from 3 to 16 individuals. The beetles were preserved in 100% ethanol and stored at −20℃. A continuous fragment (1 601 bp) of the mitochondrial cytochrome oxidase I and II (Cox1-tRNALeu-Cox2) genes was amplified using PCR, with the primers C1-J-2092 (5′-AGTTTTAGCAGGAGCAATTACTAT-3′) and TK-N-3782 (5′-GAGACCATTACTTGCTTTCAGTCATCT-3′) (Emerson *et al.*, 1999). The PCR products were purified using a TIANquick Midi Purification Kit, following the protocol's recommendations (Tiangen, Beijing, China). Sequencing reactions were carried out with the PCR primers using an ABI Prism Bigdye™ Terminator Cycle Sequencing Ready Reaction Kit on an ABI 3730XL sequencer. For the 1 601 bp fragment, a total of 882 bp were sequenced for the mtCoI gene, 63 bp for the intervening tRNA Leu gene, and 656 bp for the mtCoII gene. All sequences have been deposited in the GenBank databases under accession numbers JN600260 – JN600312.

2.2 Mitochondrial DNA variation and phylogenetic analysis

Sequences were aligned with ClustalX 1.83 (Chenna *et al.*, 2003) and double-checked manually. A matrix of 191 individual sequences was submitted into the DnaSP 4.0 software (Rozas *et al.*, 2003). The number of variable sites, the number of parsimony informative sites, the haplotype diversity (H_d) and the nucleotide diversity (P_i) were determined using the DnaSP 4.0 software (Rozas *et al.*, 2003). The phylogenetic relationships among mtDNA haplotypes were estimated using maximum likelihood (ML) analyses in PAUP * 4.0 b10 (Swofford, 2002), as well as Bayesian analyses in MrBayes 3.0 (Ronquist and Huelsenbeck, 2003). The ground beetle *Anchomenidus astur* was used as an outgroup. Two Cox1-tRNALeu-Cox2 sequences of *A. astur* were downloaded from GenBank (accession numbers: FJ173210, FJ173221). The ModelTest (Posada and Crandall, 1998) was used to find the best-fit substitution model for ML analyses, and a GTR + I + G model was applied, as determined using the Akaike information criterion. The heuristic search parameters used for the ML analysis included addition sequence of taxa with the tree bisection-reconnection (TBR) branch swapping, Multrees and Collapse options switched on. The confidence level of ML trees was accessed by 1 000 bootstrap replications. Bayesian analyses were performed with 1 250 000 generations, sampling trees every 100 generations. Likelihood values were observed with Tracer v. 1.4 (Rambaut and Drummond, 2007), discarding all the trees before stability in likelihood values as a 'burn in' (first 3 125 trees). The relationships among detected haplotypes were analyzed using Network 4.5.0.2 (Polzin *et al.*, 2003), and the median-joining network of haplotypes was constructed.

2.3 Population structure and differentiation analysis

The partition of genetic diversity within and among the populations was analyzed by analysis of molecular variance (AMOVA) (Excoffier *et al.*, 1992), using Arlequin 3.0 (Excoffier *et al.*, 2005) with 1 000 permutations.

The spatial genetic structure of haplotypes was analyzed using the program SAMOVA 1.0 (Dupanloup *et al.*, 2002, http://web.unife.it/progetti/genetica/Isabelle/samova.html) with 1 000 permutations. This program implements a simulated annealing approach to define population groups (K). In the analysis, K varied from 2 to 24 with each simulation. The number of initial conditions was set to 100. The K value with the highest F_{CT} represents the best number of groups and the best population configuration. This program implements an approach to define groups of populations that are geographically homogeneous and maximally differentiated from each other. The method is based on a simulated annealing procedure that aims to maximize the proportion of total genetic variance due to differences between groups of populations (F_{CT}). The simulated annealing process was repeated 100 times. The configuration with the largest F_{CT} value among the 100 tested was retained as the best grouping of populations. Furthermore, the parameters of population diversity (H_S, H_T) and differentiation (G_{ST}, N_{ST}) were estimated following the methods described by Pons and Petit (1995, 1996), using PERMUT (http://www.pierroton.inra.fr/genetics/labo/Software/PermutCpSSR/index.html). PERMUT computes measures of diversity and differentiation from haploid population genetic data. When a measure of the distance between haplotypes was available, we tested whether the differentiation and diversity measures differed from the equivalent measures, which do not take the

distances between haplotypes into account (*i. e.*, all haplotypes are considered equally divergent) (http://www. pierroton. inra. fr/genetics/labo/Software/ PermutCpSSR). Two different parameters (G_{ST} and N_{ST}) were compared using a permutation test with 10 000 permutations. If the N_{ST} value is significantly higher than the G_{ST} value, then the presence of phylogeographic structure can be assumed (Pons and Petit, 1996).

2.4 Demographic history analysis

Neutrality tests were implemented in Arlequin 3.0 (Excoffier *et al.*, 2005), and Fu's F_S test (Fu, 1997) and Tajima's D test (Tajima, 1989) were used to detect evidence of recent population expansion within each inferred clade, for which negative values are expected (Schneider and Excoffier, 1999). Mismatch analyses of Cox1-tRNALeu-Cox2 sequences were performed using the program Arlequin 3. 0 to explore the demographic history of the studied populations, and the parameters of population expansion were estimated. A recent growth is expected to generate a unimodal distribution of pairwise differences between sequences (Rogers and Harpending, 1992). The validity of the expansion model was tested using the sum of squared deviations (*SSD*) and Harpending's raggedness index (R) between the observed and expected mismatches.

3 RESULTS

3.1 Mitochondrial DNA variation

The results demonstrated that 45 positions were polymorphic sites, composed of 15 parsimony informative sites and 30 singleton variable sites. These polymorphic sites defined 53 haplotypes within the 191 individuals sampled from 24 localities (Table 1, Fig. 1). Of the total haplotypes, 34.29% were common haplotypes and 65.71% were private haplotypes with lower frequency (Table 1). The common haplotype T19 had a very high frequency, existing in 23 out of the 24 sampled populations. The haplotype diversity (H_d) for all sampled specimens was 0. 769, and the total nucleotide diversity (P_i) was 0. 0033. The values for the two parameters were zero in the 17th and 23rd sampling locations because only one haplotype was found at both locations (Table 1). Transitions were more frequent than transversions (ti/tv: 2.833).

Table 1　Sampling locations, sample size, GPS coordinates, and Cox1-tRNALeu-Cox2 haplotypes of *Dolichus halensis*

Population no.	Sampling locations	GPS coordinates		Elevation (m)	SS	Haplotypes (number of individuals)	P_i	H_d
		Latitude (°N)	Longitude (°E)					
1	Linxia, Gansu	35.49	102.99	2 180	9	T19(8), T26(1)	0.0001	0.222
2	Zhangxian, Gansu	34.81	104.15	2 432	4	T9(3), T22(1)	0.0006	0.5
3	Zhangxian, Gansu	34.59	104.5	2 357	4	T26(1), T19(3)	0.0003	0.5
4	Xihe, Gansu	33.87	105.16	1 782	5	T19(2), T9(3)	0.0004	0.6
5	Diebu, Gansu	34.06	103.32	2 378	7	T19(5), T26(1), T47(1)	0.0004	0.524
6	Zhouqu, Gansu	33.69	104.49	1 225	6	T19(3), T47(2), T26(1)	0.0005	0.733
7	Wenxian, Gansu	32.94	104.68	925	8	T27(1), T19(3), T47(4)	0.0007	0.679
8	Liangdang, Gansu	33.93	106.42	972	6	T47(4), T19(1), T15(1)	0.0005	0.6
9	Liangdang, Gansu	33.94	106.36	1 159	11	T15(1), T17(1), T19(6), T26(1), T36(1), T37(1)	0.0008	0.727
10	Lueyang, Shaanxi	33.52	105.91	715	5	T19(4), T49(1)	0.0005	0.4
11	Liuba, Shaanxi	33.54	106.92	900	6	T47(1), T48(1), T19(4)	0.0005	0.6
12	Zhouzhi, Shaanxi	33.84	107.83	1 353	16	T1(1), T8(1), T18(1), T19(9), T26(2), T36(1), T53(1)	0.001	0.692
13	Ningshan, Shaanxi	33.55	108.55	1 372	13	T2(1), T3(1), T5(1), T6(1), T7(1), T8(2), T9(1), T13(1), T17(1), T19(1), T52(1), T20(1)	0.002	0.987
14	Shiquan, Shaanxi	33.27	108.09	526	11	T19(5), T50(1), T34(5)	0.0009	0.636
15	Zhashui, Shaanxi	33.83	109.27	1 260	7	T4(1), T19(1), T20(2), T24(1), T30(1), T39(1)	0.0017	0.952

续表 1 Table 1 continued

Population no.	Sampling locations	GPS coordinates		Elevation (m)	SS	Haplotypes (number of individuals)	P_i	H_d
		Latitude (°N)	Longitude (°E)					
16	Zhenan, Shaanxi	33.24	109.17	496	5	T19(3), T23(2)	0.0004	0.6
17	Shanyang, Shaanxi	33.63	109.94	1 030	3	T19(3)	0	0
18	Luonan, Shaanxi	34.08	110.48	765	14	T9(3), T19(7), T43(1), T44(1), T45(1), T46(1)	0.0007	0.736
19	Huayin, Shaanxi	34.54	109.95	352	5	T9(1), T25(1), T43(2), T19(1)	0.0011	0.9
20	Lushi, Henan	34.25	111.01	970	15	T10(1), T11(1), T15(1), T16(1), T19(5), T29(1), T31(1), T32(1), T35(2), T38(1)	0.0018	0.895
21	Yichuan, Henan	34.42	112.43	199	5	T28(1), T19(2), T11(1), T31(1)	0.0014	0.9
22	Xixia, Henan	33.69	111.42	807	10	T12(1), T19(8), T21(1)	0.0004	0.378
23	Tanghe, Henan	32.68	112.69	113	3	T19(3)	0	0
24	Yunxi, Hubei	33.1	110.27	605	13	T9(1), T14(1), T15(1), T19(4), T33(1), T34(1), T40(1), T41(1), T42(1), T51(1)	0.0018	0.923
				Total	191	53	0.0033	0.769

P_i: Nucleotide diversity; H_d: Haplotype diversity.

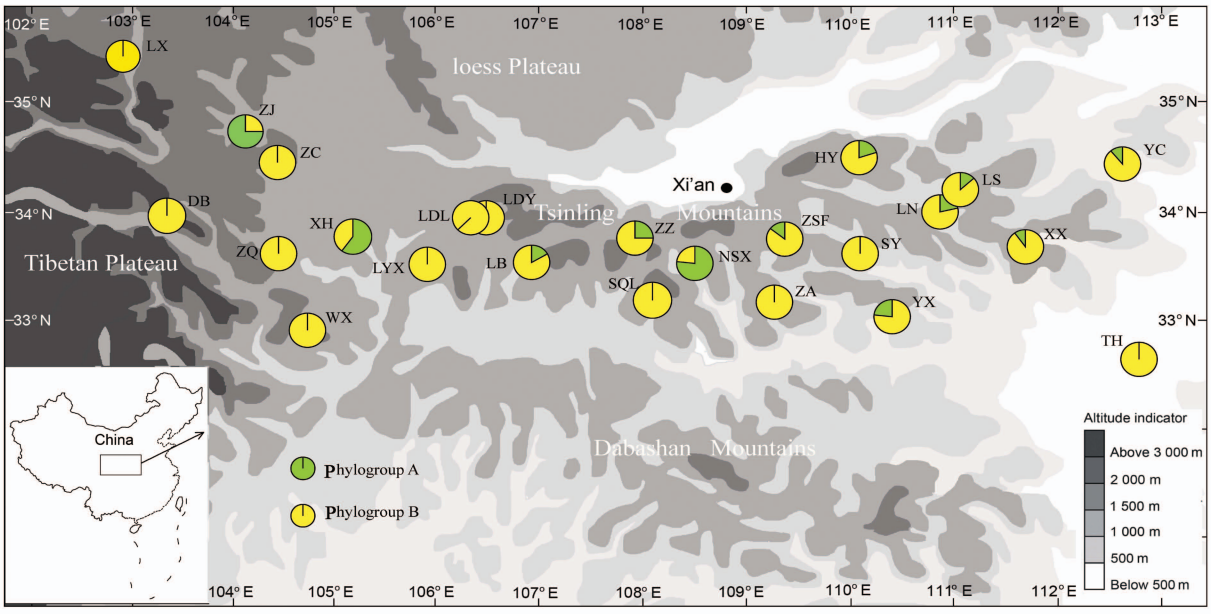

Fig. 1 Locations of sampled populations and geographical distribution of the Cox1-tRNALeu- Cox2 clades

Phylogenetic analysis (Bayesian inference) of the 53 haplotypes revealed two major clades (A and B) (Fig. 2). The ML tree was discarded due to very low bootstrap values at most nodes. Clade A, containing 35.85% haplotypes of the total (19 haplotypes), existed in 12 out of the 24 sampled populations, while clade B, containing 64.15% haplotypes of the total (34 haplotypes), existed in all the sampled populations. In the haplotype parsimonious network, five median vectors were employed, and two phylogroups were recovered (Fig. 3).

3.2 Population structure and differentiation

AMOVA analysis suggested that most of the variation occurred within populations (86.61%), while differences among populations only contributed 13.39% to the total (Table 2). For clades, the AMOVA results indicated that the percentage of variation within clades (56.70%) was higher than that among clades (43.30%) (Table 2).

The total genetic diversity H_T (0.705) among all sampled populations was higher than the average within-population diversity H_S (0.612). For the spatial

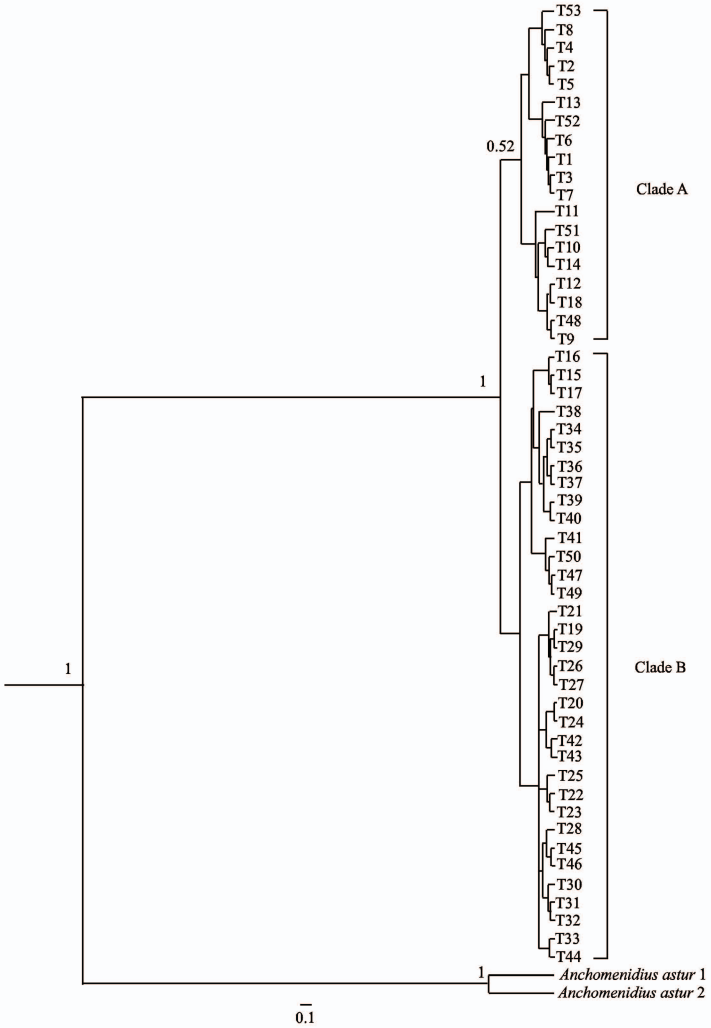

Fig. 2　Bayesian tree based on the Cox1-tRNALeu- Cox2 haplotypes

Numbers beside nodes represent the Bayesian posterior probability. The Bayesian posterior probability of nodes below 0.3 is not marked.

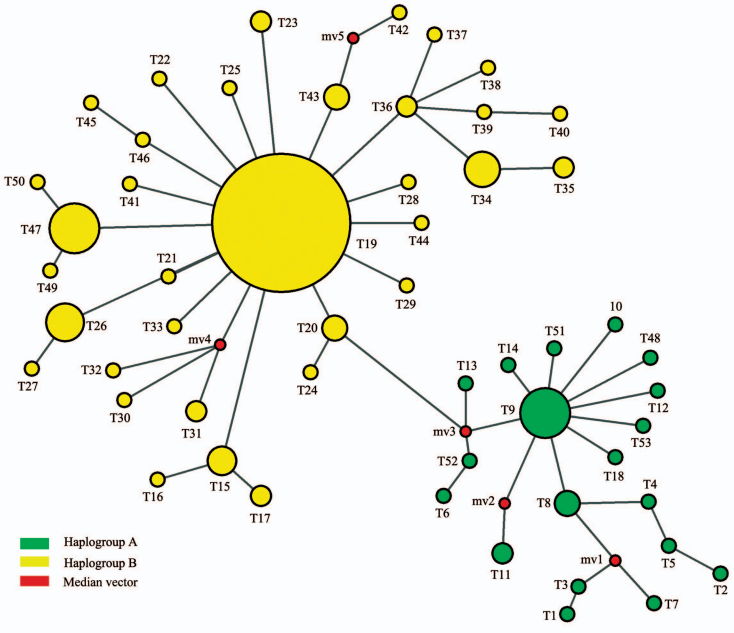

Fig. 3　Parsimonious network of the Cox1-tRNALeu-Cox2 sequences

Each haplotype is represented by a circle, with the area of the circle proportional to its frequency. Samples from individual haplogroups and median vectors (mv1 -5) are indicated by different colors.

analysis of molecular variance, we used K values increasing from 2 to 24, and found moderately low F_{CT} values that steadily fluctuated from $F_{CT}=0.204$ (when $K=7$) to $F_{CT}=0.367$ (when $K=2$). Thus, the SAMOVA tests failed to reveal any meaningful phylogeographic structure. Furthermore, the results of the PERMUT analyses detected no phylogeographic structure of the sampled populations based on the mtDNA haplotype data because the total N_{ST} value (0.154) was not significantly higher than the G_{ST} value (0.132; $P=0.085$).

3.3 Demographic history

The results of the neutrality tests on the total population and the two mtDNA clades indicated that both the Tajima's D and Fu's Fs tests resulted in significantly negative values (Table 3). Mismatch distribution analyses showed a unimodal frequency distribution of pairwise difference in the total population and in each clade. Neither the sum of squared deviations (*SSD*) (except *SSD* of clade B) nor Harpending's raggedness index (R) test reject the hypothesis of a sudden expansion model (Table 3, Fig. 4).

Table 2 Results of the analysis of molecular variance (AMOVA) of *Dolichus halensis*

Source of variation	*d.f.*	Sum squares	Variance components	Percentage of variation	Fixation index (F_{ST})
Among populations	23	38.796	0.11761 V_a	13.39	0.13393 **
Within population	167	127.01	0.76054 V_b	86.61	
Total	190	165.806	0.87815		
Among clades	1	29.958	0.54881 V_a	43.3	0.43296 **
Within clade	189	135.848	0.71877 V_b	56.7	
Total	190	165.806	1.26758		

d.f.: Degree of freedom; F_{ST}: Correlation within populations relative to the total; **: $P<0.01$.

Table 3 Mismatch distribution analyses and neutrality test of *Dolichus halensis*

Clades	N	n	τ (CI = 95%)	θ_0	θ_1	*SSD* (*P* value)	R (*P* value)	Tajima's D (*P* value)	Fu's F_s (*P* value)
Clade A	32	19	2.59	0.047	10.852	0.003	0.025	−1.579 *	−13.251 **
			(0.58 − 3.77)			(*P* = 0.75)	(*P* = 0.86)	(*P* = 0.04)	(*P* = 0.00)
Clade B	159	34	0.93	0	99 999	0.011	0.031	−2.372 **	−28.782 **
			(0.68 − 1.25)			(*P* = 0.04)	(*P* = 1.00)	(*P* = 0.00)	(*P* = 0.00)
Total	191	53	1.15	0.803	7.583	0	0.019	−1.291 *	−27.633 **
			(0.00 − 3.61)			(*P* = 0.98)	(*P* = 0.95)	(*P* = 0.001)	(*P* = 0.00)

N: Number of sequences; n: Number of haplotypes; τ: Time in number of generations elapsed since the sudden expansion episode; θ_0: Pre-expansion population size; θ_1: Post-expansion population size; *SSD*: Sum of squared deviations; R: Raggedness indices; *: $P<0.05$; **: $P<0.01$.

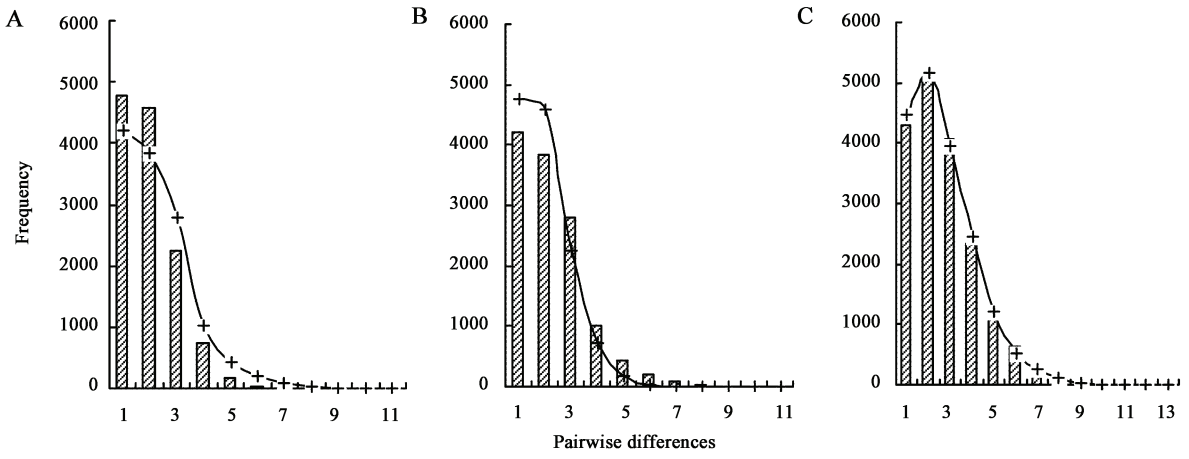

Fig. 4 Mismatch distribution analysis for clades A (A) and B (B) and the total population (C)

The histograms represent the observed frequencies of pairwise differences among haplotypes, and the line shows the curve expected for an expanded population.

4 DISCUSSION

It is difficult to determine the origin center or the glacial refugium for the Qinling Mountains population because most local populations show high genetic diversity. The high level of habitat diversity in the Qinling Mountains is most likely one of the main factors leading to the high genetic diversity of the total population and of most local populations.

AMOVA analysis showed significant genetic differentiation within populations. However, the low genetic variation among the local populations affirmed the lack of phylogeographic structure within the Qinling Mountains population of this species.

Individuals from one population were distributed across different clades, and conversely, one clade contains individuals from different populations. For example, both clades appear to be distributed across the whole range of studied populations (Figs. 1, 2). Therefore, there is no correlation between the phylogroups and their geographical distributions. Furthermore, neither the SAMOVA test nor the PERMUT analysis indicated significant phylogeographic structure. This absence of phylogeographic structure is in contrast to the studies on the phylogeography of other ground beetles such as *Leptocarabus seishinensis*, *L. semiopacus* and *L. koreanus* (Zhang *et al.*, 2006).

China experienced at least four glacial periods in the Quaternary period, and the last glaciation took place 0.07 Myr ago (Sun *et al.*, 1977). The founder effects during the postglacial population recovery lead to a rapid population expansion (Lessa *et al.*, 2003), which may subsequently erase the previously formed phylogeographic structure.

Both the total population and most local populations have high haplotype diversity and low nucleotide diversity, implying that they most likely have undergone population expansion after a period of low effective population size. Both clades with a star-like phylogeny encircling the basic haplotypes with a wide distribution (Fig. 3) indicated an exponential expansion (Avise, 2000). The unimodal mismatch frequency distribution pattern based on the mtDNA sequence was fairly consistent with the predicted distribution under a model of population expansion (Fig. 4) (Rogers and Harpending, 1992). All mismatch distributions of the total population and the two clades that formed sharp peaks implied a smaller initial population prior to the expansion or bottleneck effect (Fig. 4) (Rogers and Harpending, 1992). Both Tajima's D and Fu's F_S values are sensitive to bottleneck effects and population expansion, and they become more negative under these circumstances (Tajima, 1993, 1996; Aris-Brosou and Excoffier, 1996; Martel *et al.*, 2004), although Fu's F_S is more sensitive to recent population growth (Fu, 1997). Meanwhile, neither the sum of squared deviations (*SSD*) nor Harpending's raggedness index (R) test can reject the hypothesis of a sudden expansion model. A population with high h value ($H_d > 0.5$) and low π ($P_i < 0.005$ or 0.5%) indicates that it experienced a bottleneck effect (Grant and Bowen, 1998). On the other hand, high H_d values and lower P_i values indicate that the time after population expansion is long enough to examine the change of haplotypes resulting from mutation but not long enough to accumulate large differences among sequences (Avise, 2000). Together, the above analyses confirmed the existence of a postglacial population expansion in *D. halensis*. The retreat of the last glaciation that took place in China (Sun *et al.*, 1977) might have provided the opportunity for this population expansion. These results are similar to those obtained from the ground beetle *Pheropsophus jessoensis* (Li *et al.*, 2012), thus providing further evidence for high genetic diversity in ground beetles that inhabit mountainous areas and for postglacial population expansion that may occur amongst phylogenetically close species in these areas.

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秦岭地区赤胸梳爪步甲种群的遗传多样性和扩张历史分析

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摘要：赤胸梳爪步 *Dolichus halensis*（Schaller）（鞘翅目：步甲科）是重要的捕食性天敌昆虫，在我国分布广泛。为揭示其种群遗传多样性和扩张机制，本研究以秦岭地区为中心，以线粒体 Cox1-tRNALeu-Cox2 基因片段为分子标记，对来自于 24 个采集点共 191 个个体进行了检测分析。在长度为 1 601 bp 的碱基中共检测到 45 个变异位点，定义了 53 个单倍型，单倍型多样性高（H_d = 0.796），而核苷酸多样性较低（P_i = 0.0033）。系统发育分析结果表明该地区该物种存在两大进化枝。分子变异分析（AMOVA）表明 86.61% 的变异来源于种群内。SAMOVA 和 PERMUT 分析结果一致，表明秦岭地区分布的赤胸梳爪步甲种群不存在明显的谱系地理结构。中性检验和错配分布分析的结果一致，表明该物种在秦岭地区曾经发生过种群扩张。综上，认为赤胸疏爪步甲种群经历过冰期后的扩散。

关键词：赤胸疏爪步甲；线粒体 DNA；遗传多样性；种群扩张；秦岭

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